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LOCOMOTOR PERFORMANCE OF *DROSOPHILA MELANOGASTER*: INTERACTIONS AMONG DEVELOPMENTAL AND ADULT TEMPERATURES, AGE, AND GEOGRAPHY

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Abstract.—We explored the extent to which a phenotypic trait (walking speed) of *Drosophila melanogaster* is influenced by population, developmental temperature, adult temperature, and age. Our goals were to estimate the importance of these factors and to test the beneficial acclimation hypothesis. We measured speed of flies from two populations (the Congo and France) that developed at different temperatures (18, 25, and 29°C) and were tested at different temperatures (18, 25, and 29°C) and ages (2, 7, 13 days). Not surprisingly, speed increased strongly with test temperature. Speed was generally greatest for flies reared at an intermediate developmental temperature, contrary to the beneficial acclimation hypothesis, which predicts that speed would be greatest when influenced by interactions involving population. For example, speed was greatest for flies from France that developed at a low temperature, but for flies from the Congo that developed at a high temperature. The impact of developmental temperature declined with age. Surprisingly, speed actually increased with age for flies raised and maintained at a low temperature, but decreased with age for flies raised and maintained at an intermediate or at a high temperature. Thus, walking performance is highly dynamic phenotypically, complicating potential attempts to predict responses to selection on performance.

Key words.—Acclimation, aging, beneficial acclimation hypothesis, *Drosophila melanogaster*, performance, speed, temperature.

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Natural selection acts on phenotypes. However, predicting evolutionary responses to selection can be complicated, especially for physiological performance traits. Such traits are not fixed but change dynamically in response to interactions involving an individual's genotype and its prior and current environment (Gupta and Lewontin 1992; Schlichting and Pigliucci 1998), as well as its age (Medawar 1952; Rose 1991). Although many studies have explored how each of these factors individually influences norms of reactions, far fewer have determined the interactions involving two or more of these factors. Here we describe an experiment designed to elucidate the simultaneous influence of four factors on the physiological performance of *Drosophila melanogaster*.

We compared flies from a tropical versus a temperate-zone population, reared them at three different temperatures, and tested their walking speed as adults at three different temperatures and at three different ages. This factorial design (two populations × three developmental temperatures × three adult temperatures × three adult ages) enables us to explore not only the sensitivity of performance to each factor individually, but also to their interactions. For example, we can determine whether the impact of developmental temperature on adult performance decays with age; such a decay might be expected as a fly acclimates to its particular adult environment (see Nunney and Cheung 1997).

We selected walking speed as the focal physiological trait. Walking speed is a convenient index of overall physiological performance, and it is directly correlated with mating success in male *D. melanogaster* (e.g., in dominance interactions, Partridge et al. 1987; Gilchrist et al. 1997; Wisco et al. 1997). Thus, an understanding of genotypic and environmental factors influencing walking speed should be ecologically and evolutionarily relevant to flies. We manipulate temperature because in *Drosophila,* as in other ectotherms, temperature is generally the most important environmental factor helping to explain the geographic distribution, abundance, and performance of species (David et al. 1983).

Our experimental design also enables us to test a classical physiological hypothesis that performance is maximal for flies tested as adults in the environment in which they were reared. This hypothesis, which is now called the beneficial acclimation hypothesis (Leroi et al. 1994), is widely accepted in the physiological literature. Nevertheless, several recent studies challenge its generality (Zwaan et al. 1991; Leroi et al. 1994; Zamudio et al. 1995; Padilla and Adolph 1996); and several competing hypotheses have been proposed (see reviews in Huey and Berrigan 1996; Huey et al. 1999). For example, the optimal developmental temperature hypothesis posits that flies that are reared at intermediate temperatures will be vigorous as adults and will perform well in a variety of adult environments, not just the one in which they were reared (Cohet and David 1978; Zamudio et al. 1995). Using recently developed statistical approaches, we can directly test these and other competing hypotheses (see Huey et al. 1999).

Overall, our experiments are designed to explore the dynamics of phenotypic plasticity in a physiological performance trait. We show that reaction norms for locomotor performance are indeed complex, implying that any attempt to predict evolutionary responses to selection on speed will necessarily be difficult.

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MATERIALS AND METHODS

Experimental Design

Large samples of *D. melanogaster* were collected from Bordeaux (France, 45°N) in December 1997 and in Pointe Noire (the Congo, 4.5°S) in September 1998. Flies were kept in bottles as laboratory mass cultures at 20°C and 16:8 L:D (~200 pairs per generation; cornmeal, sugar, yeast, agar, Nipagine medium) and tested in January 1999. Thus, flies from the Congo had been in captivity for fewer months than had the flies from France (4 vs. 13 months) prior to testing. (The lines undoubtedly experienced selection for convergence to a common laboratory environment during this period.)

Successful development in *D. melanogaster* occurs between 12°C and 32°C (David et al. 1997). We selected three developmental temperatures (18, 25, and 29°C) that cover most of the range and that are not pathological. After allowing parents to oviposit for 4–6 h at 21°C, we transferred sets of 50 eggs into vials (six vials per developmental temperature per population per block) and then assigned each vial a developmental temperature. After emerging, flies were anaesthetized briefly with CO_2, sexed, and segregated. We used males not only to minimize variation in speed related to sex and size (see Gilchrist et al. 1997), but also because a male's speed influences his mating success (Partridge et al. 1987).

Within one day of eclosing, adult males from all six vials for each treatment were mixed (to dilute any vial effect), then transferred to fresh vials, and maintained at one of three adult temperatures (18, 25, or 29°C). We tested their locomotor performance (below) at 2, 7, or 13 days of age (13 days brackets the life expectancy of flies in nature; cf. Rosewell and Shorrocks 1987). For each treatment, we measured speeds of 15 males in each of two blocks (blocking was necessary for logistical reasons). Thus, we measured 1600 flies total.

Measuring Walking Speed

We measured adult walking speed by placing a single adult into a test tube and knocking the fly to the bottom of the tube (details in Gilchrist 1996). The fly would then walk quickly up the tube, and we recorded the time for it to walk from the bottom to the top (distance = 8 cm). We immediately reran each fly and analyzed its average speed.

Data Analysis

Data were analyzed by using an ANOVA for ordered factors, which takes into account the ordering inherent in temperature and in age (Huey et al. 1999). The appropriate single-degree-of-freedom contrasts for ordered factors with three levels are linear and quadratic (Sokal and Rohlf 1981). The blocks did not differ significantly by ANOVA or *t*-test and therefore were pooled. Distributions were nearly normal and thus not transformed.

The formal statistical model is based on one in Huey et al. (1999) and is an ANOVA for speed (V_m) as a function of developmental temperature (both linear ($T_{dev(l)}$) and quadratic ($T_{dev(q)}$) contrasts), adult test temperature (T_{test}), and their interactions. For implementation here, we added population (fixed effect) and age (both linear and quadratic contrasts). Because essentially all higher-order interactions were insignificant and minor, we report only the main effects and two-way interactions.

TABLE 1. Least squared means ± SE for velocities (cm/sec) computed for main effects. Sample sizes are in parentheses. One column shows values for the entire dataset; the other shows values for the subset of the data where development temperature equals test temperature. Values with different superscripts are significantly different at $P > 0.05$. For population, the means were tested by ANOVA. For T_{dev} and age, differences were tested using Tukey's method for simultaneous confidence intervals.

Factor	Full dataset	$T_{dev} = T_{test}$
Population		
Congo	2.00 ± 0.016^{a} (810)	2.11 ± 0.029^{a} (270)
France	2.00 ± 0.017^{a} (810)	1.99 ± 0.029^{b} (270)
T_{test} (°C)		
18	1.44 ± 0.017^{a} (540)	1.48 ± 0.029^{a} (180)
25	2.23 ± 0.021^{b} (540)	2.35 ± 0.041^{b} (180)
29	2.33 ± 0.022^{c} (540)	2.30 ± 0.035^{b} (180)
T_{dev} (°C)		
18	2.00 ± 0.020^{a} (540)	—
25	2.09 ± 0.022^{b} (540)	—
29	1.91 ± 0.018^{c} (540)	—
Age (days)		
2	2.04 ± 0.021^{a} (540)	2.11 ± 0.035^{a} (180)
7	2.04 ± 0.019^{a} (540)	$2.05 \pm 0.034^{a,b}$ (180)
13	1.93 ± 0.021^{b} (540)	1.99 ± 0.036^{b} (540)

RESULTS

Main Effects

Least-squared means of walking speed for the main effects are given in Table 1 (full dataset), with specific levels of each effect tested by Tukey's method. Associated ANOVA results are presented in Table 2.

Flies from the Congo and France had essentially identical speeds (Tables 1, 2). In contrast, flies at different test temperatures had very different speeds (Tables 1, 2, $P < 0.001$): The warmer the temperature, the faster the flies. Developmental temperature also had major effects: Walking speed was highest for flies that developed at intermediate temperature (25°C), and lowest for flies reared at 29°C (Table 1). This pattern is reflected in the highly significant quadratic contrast (Table 2, $P < 0.001$) and in the weakly significant ($P < 0.05$) linear (and negative) contrast.

Two- and 7-day-old flies had identical speeds, but 13-day-old flies walked significantly more slowly (Tables 1, 2). Both the linear and quadratic contrasts for age are significant; but the linear effect, which was negative, was stronger (Table 2).

Because adult flies were maintained and then raced at the same temperature, the above age effect may be confounded by an acclimation effect as adults adjust physiological to a new temperature environment that differs from their developmental one. To determine whether age itself does have direct effects, we analyzed walking speeds only of those flies that had an unchanged developmental and adult test temperature (see $T_{dev} = T_{test}$ in Table 1). Speed of these flies still

TABLE 2. Analysis of variance of walking speed in *Drosophila melanogaster.* Developmental temperature and age are treated as ordered factors, with orthogonal polynomial contrasts provided in italics below the main effect and interaction terms. Pop, population; T_{test}, adult test temperature; T_{dev}, developmental temperature; (*l*), linear contrast; (*q*), quadratic contrast.

Factor	df	MS	*F*-value
T_{test}	2	127.07	681.33***
T_{dev}	2	3.98	21.36***
(*l*)	1	1.18	6.33*
(*q*)	1	6.79	36.39***
Age	2	2.33	12.49***
(*l*)	1	3.74	20.05***
(*q*)	1	0.92	4.92*
Pop × T_{test}	2	0.25	1.33^{ns}
Pop × T_{dev}	2	3.47	18.61***
(*l*)	1	5.88	31.52***
(*q*)	1	1.06	5.70*
Pop × Age	2	0.91	4.88**
(*l*)	1	1.45	7.76**
(*q*)	1	0.37	1.99^{ns}
T_{test} × Age	4	11.74	62.93***
(*l*)	2	20.41	109.44***
(*q*)	2	3.06	16.43***
T_{test} × T_{dev}	4	0.47	2.52*
(*l*)	2	0.43	2.31^{ns}
(*q*)	2	0.51	2.73^{ns}
T_{dev} × Age	4	1.30	6.95***
(*l*) × (*l*)	1	0.03	0.18^{ns}
(*q*) × (*l*)	1	0.86	4.61*
(*l*) × (*q*)	1	4.28	22.96***
(*q*) × (*q*)	1	0.01	0.04^{ns}
Residuals	1594	0.19	

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ns, nonsignificant.

declined linearly with age ($P < 0.01$), suggesting that the age per se is responsible for the senescent decline in speed.

Interactions

Population was not significant as a main effect (above), but neverthless had several significant interactions. For example, population interacted with developmental temperature: Flies from France were fastest if they had developed at low (18°C) temperature, whereas flies from the Congo were fastest if they developed at intermediate (25°C) temperature (Fig. 1). In addition, population showed small but significant interactions with age (Table 2): Flies from France walked more slowly as they aged, whereas flies from the Congo walked fastest at 6 days of age (data not shown). Interactions between adult test temperature and age were significant (Table 2). At 29°C, speed decreased with age, whereas at 18°C, speed actually increased with age (Fig. 2).

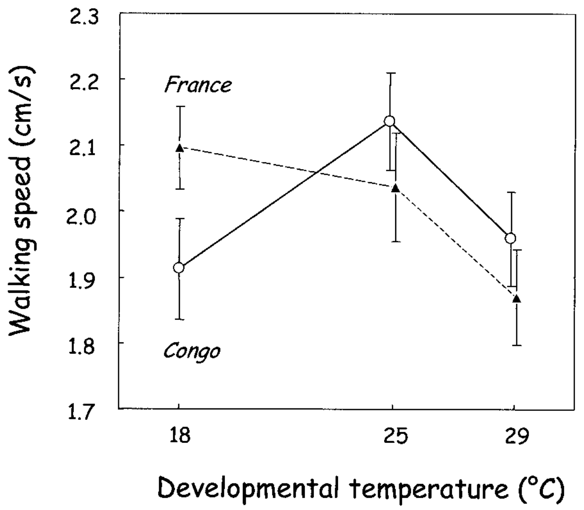

FIG. 1. Effect of population and of developmental temperature on walking speed of *Drosophila melanogaster.* Each mean (±95% confidence interval) represents the average of 270 values (30 flies × three adult temperatures × three ages).

Two of the four interactions between the linear and quadratic effects of age and of developmental temperature were significant. The interaction between the linear effect of developmental temperature and the quadratic effect of age was strong but difficult to interpret. To clarify this interaction and to examine more closely whether the impact of developmental temperature declined as a fly aged, we did separate ANOVAs for each age (data not shown). One might expect that the influence of developmental temperature will decline with age, as the adults acclimate to their new environment. For 2-day-old flies, both the linear and quadratic terms for developmental temperature indeed had strong impacts on speed ($P < 0.001$ in both cases). For 7-day-old flies, only the quadratic effect of developmental temperature remained significant ($P < 0.001$). For 13-day-old flies, neither the linear or quadratic effect of developmental temperature was significant ($P > 0.06$ in both cases). Thus, the impact of developmental temperature decayed as the flies aged.

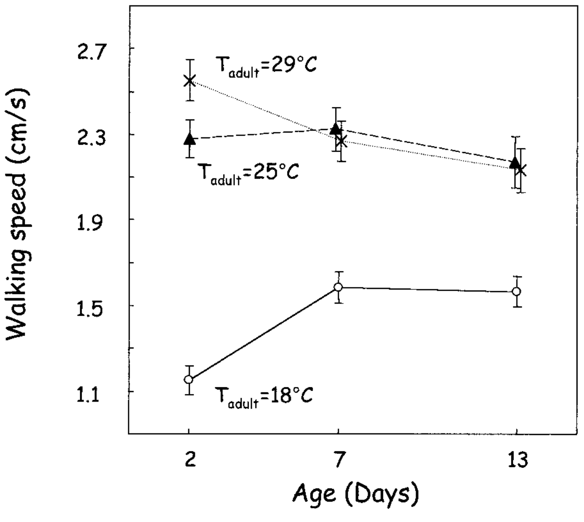

FIG. 2. Effect of age on walking speed for *Drosophila melanogaster* from the Congo maintained at three different adult temperatures (developmental temperatures are combined). Each mean (±95% confidence interval) represents the average of speeds for 90 flies (30 flies × three developmental temperatures). Flies from France show a similar pattern (data not shown).

DISCUSSION

Our primary goal was to evaluate direct and interactive effects of several factors that potentially influence the plasticity of a physiological trait. Our analyses show that phenotypic effects (e.g., adult and developmental temperatures) have the largest impact on a physiological phenotype, whereas population of origin has only minor effects. Of particular interest are strong interactions among factors, implying that reaction norms for performance traits are highly complex.

Adult test temperature had by far the biggest impact on walking speed (Table 1): Flies at 25°C walked 61% faster on average than did flies at 18°C. The temperature sensitivity of walking speed (Crill et al. 1996; Gilchrist et al. 1997) and other traits is well established in *Drosophila* (David et al. 1983, 1998; Zwaan et al. 1991; Zamudio et al. 1995; Nunney and Cheung 1997; Huey et al. 1999; Lehmann 1999) and many other ectotherms (Johnston and Bennett 1996).

Developmental temperature also had a significant impact on walking speed (see also Crill et al. 1996), as has previously been shown for many other traits in *Drosophila* (David et al. 1983; Crill et al. 1996; Nunney and Cheung 1997). Overall, flies developing at intermediate temperatures walked 9% faster than did flies developing at 29°C (Table 1). Age is also significant, and 13-day old flies were about 5% slower than 2- or 7-day-old flies (Table 1).

Athough geographical origin (the Congo vs. France) was not significant as a main effect (Tables 1, 2), it was involved in several significant interactions. For example, flies from France walked fastest if they developed at low temperatures, whereas flies from the Congo walked faster if they developed at intermediate temperatures—a pattern that is seemingly consistent with a hypothesis of adaptation to local developmental temperature (presumably warmer in the Congo than in France).

Our data are also relevant to a test of the beneficial acclimation hypothesis (see Leroi et al. 1994), which predicts that individuals acclimated to (or that develop in) one environment perform better in that environment than do individuals acclimated to a different one. This hypothesis is long been assumed as a truism in the physiological literature (e.g., Leroi et al. 1994; Huey and Berrigan 1996). Even so, several competing hypotheses have been proposed (see introduction; Cohet and David 1978; Huey and Berrigan 1996; Huey et al. 1999). Our factorial design lends itself to a formal statistical model that simultaneously tests all competing hypotheses (see Huey et al. 1999).

We found no support for beneficial acclimation, which predicts that flies would walk fastest at temperatures matching their developmental temperature. Instead we found significant support for the optimal developmental temperature hypothesis (Cohet and David 1978): Flies reared at intermediate temperature ran relatively quickly at *all* adult temperatures, not just at their rearing temperature (Table 1). In other words, flies reared at intermediate temperatures are seemingly robust and will likely have high fitness in diverse adult environments. This hypothesis has also received support in a recent analysis of patterns for diverse taxa and diverse traits and has important implications for understanding performance in nature (Huey et al. 1999). Nevertheless, support for beneficial acclimation does exist in some studies (Nunney and Cheung 1997; Huey et al. 1999), so the impact of developmental temperature may be difficult to predict.

Interestingly, the influence of developmental temperature on performance decays as a fly ages (Table 2), even for the subset of flies in which test and developmental temperatures were unchanged (see above). Nunney and Cheung (1997) similarly found that developmental temperature influenced early fecundity of *D. melanogaster,* but not on lifetime fecundity. In effect, age increasingly buffers developmental effects.

The effect of age is further complicated by a significant interaction with adult temperature. Again, consider those flies for which adult and developmental temperatures are the same. For flies at intermediate or high temperature, speed declined with age; but for flies at low temperature, speed actually increased with age (Fig. 2)—hardly a pattern of senescence. Perhaps flies that develop and live at a low temperature are physiologically less mature at eclosion (and thus have delayed onset of senescence) than flies that develop and live at a higher temperature.

In summary, the physiological performance of adult flies was strongly and dynamically influenced by several ontogenetic factors, often involving interactions among factors. The observed complexity and dynamics of these patterns reinforce a general view that predicting evolutionary responses of physiological phenotypes selection will be very difficult.

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PARENTAL AND DEVELOPMENTAL TEMPERATURE EFFECTS ON THE THERMAL DEPENDENCE OF FITNESS IN *DROSOPHILA MELANOGASTER*

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Abstract.—Cross-generational effects refer to nongenetic influences of the parental phenotype or environment on offspring phenotypes. Such effects are commonly observed, but their adaptive significance is largely unresolved. We examined cross-generational effects of parental temperature on offspring fitness (estimated via a serial-transfer assay) at different temperatures in a laboratory population of *Drosophila melanogaster*. Parents were reared at 18°C, 25°C, or 29°C (T_{par}) and then their offspring were reared at 18°C, 25°C, or 29°C (T_{off}) to evaluate several competing hypotheses (including an adaptive one) involving interaction effects of parental and offspring temperature on offspring fitness. The results clearly show that hotter parents are better; in other words, the higher the temperature of the parents, the higher the fitness of their offspring, independent of offspring thermal environment. These data contradict the adaptive cross-generational hypothesis, which proposes that offspring fitness is maximal when the offspring thermal regime matches the parental one. Flies with hot parents have high fitness seemingly because their own offspring develop relatively quickly, not because they have higher fecundity early in life.

Key words.—Cross-generational effects, developmental temperature, *Drosophila melanogaster*, fitness, maternal effects.

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Cross-generational (parental) effects are a type of phenotypic plasticity in which the environment of parents has nongenetic influences on the phenotypes of their offspring. Parental effects are important evolutionarily not only because they influence short-term responses to selection (Falconer 1989; Kirkpatrick and Lande 1989; Riska 1989), but also because they are potentially adaptive (Mousseau and Dingle 1991; Rossiter 1996; Fox et al. 1997). For example, if the parental environment is correlated with that of their offspring, then parents could enhance their own fitness by activating developmental programs that tune their offspring's phenotype for that environment (Fox et al. 1997; Donohue and Schmitt 1999).

Many recent studies have shown that parental environ-

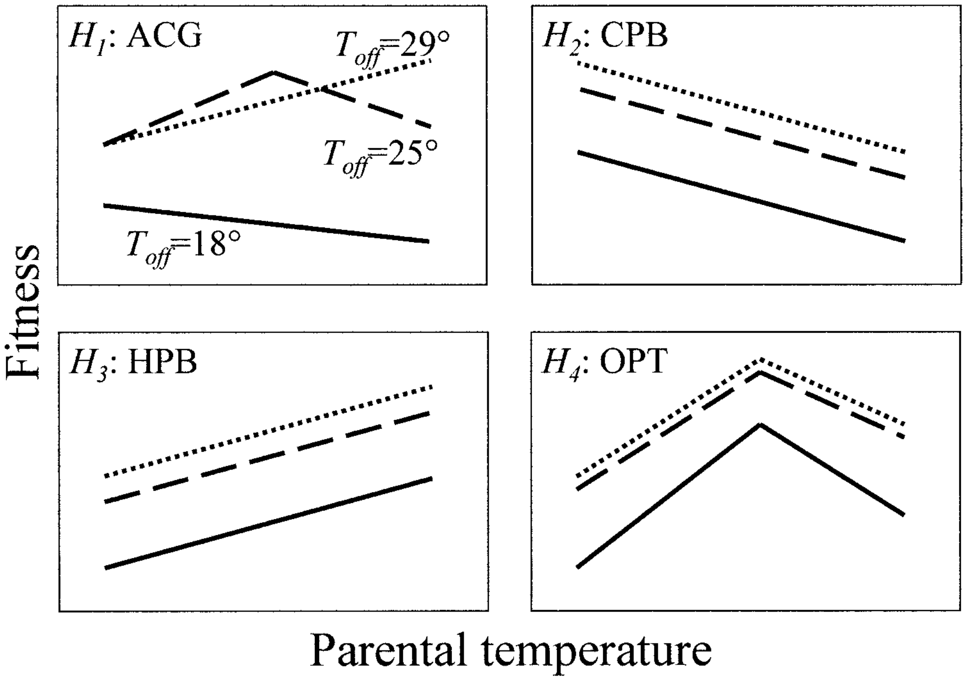

FIG. 1. Graphical representations of the adaptive cross-generational (ACG), colder parents are better (CPB), hotter parents are better (HPB), and optimal parental temperature (OPT) hypotheses.

ments have important phenotypic effects on offspring, and several studies show that parental effects often enhance life-history or physiological correlates of offspring fitness (reviews in Rossiter 1996; Mousseau and Fox 1998). To our knowledge, however, no study of a eukaryotic organism has yet examined whether parental effects actually enhance offspring fitness itself, as estimated by the per capita rate of population increase.

Here we consider the effect of parental temperature on the thermal dependence of offspring fitness in a laboratory population of *Drosophila melanogaster*. Temperature is a key environmental variable for ectotherms (David et al. 1983; Hoffmann and Parsons 1991), and parental temperature has diverse phenotypic effects on the offspring in *D. melanogaster* (Zamudio et al. 1994; Crill et al. 1996; Huey and Berrigan 1996). Whether these responses actually increase fitness in this species, however, is unknown. Evolutionary physiologists have long assumed that acclimation responses within a generation are adaptive, a view codified by Leroi et al. (1994, p. 1917) as the beneficial acclimation hypothesis: ''acclimation to a particular environment gives an organism a performance advantage over another organism that has not had the opportunity to acclimate to that particular environment.'' Recently, however, several researchers have challenged that assumption with new evidence and proposed several alternative hypotheses based on patterns observed in laboratory experiments (reviewed in Huey et al. 1999).

In this paper, we modify the statistical methods we recently developed to test the beneficial acclimation hypothesis (Huey and Berrigan 1996) to a set of related hypotheses concerning cross-generation acclimation. We use a factorial experimental design that enables us to measure the fitness of offspring as a function of interactions involving parental and offspring temperature. We then use the resulting data to test several competing, nonexclusive hypotheses (below) that are derived from hypotheses of the effects developmental temperature on adult (i.e., within-generation) fitness.

Competing hypotheses are diagramed in Figure 1. The adaptive cross-generational (ACG) hypothesis proposes that offspring reared and living in the same environment as that of their parents will have higher fitness than will offspring living in an environment different from that of their parents. This hypothesis is a cross-generational version of the beneficial acclimation hypothesis (Leroi et al. 1994; Huey and Berrigan 1996) or a temperature version of a general adaptive maternal effect hypothesis (Mousseau and Fox 1998). The colder parents are better (CPB) hypothesis proposes that parents reared under cool temperatures produce offspring that are invariably more fit than do parents reared under warm conditions. For example, parents reared under cool temperatures will be large and therefore will produce large offspring (Crill et al. 1996), with attendant fitness advantages. The hotter parents are better (HPB) hypothesis is just the opposite: Parents that develop under hotter temperatures will produce more fit offspring than parents grown under cooler temperatures. This hypothesis might apply if small size confers a fitness advantage through enhanced motility or an earlier age at first reproduction (McLachlan and Allen 1987). The optimum parental temperature (OPT) hypothesis proposes that parents living at intermediate temperatures produce more fit offspring than do parents living at more extreme high or low temperatures. This hypothesis derives from the intermediate developmental temperature hypothesis of Cohet and David (1978), who noted that development at intermediate temperatures produced flies with a seemingly well-integrated physiology that perform well under a variety of thermal condi-

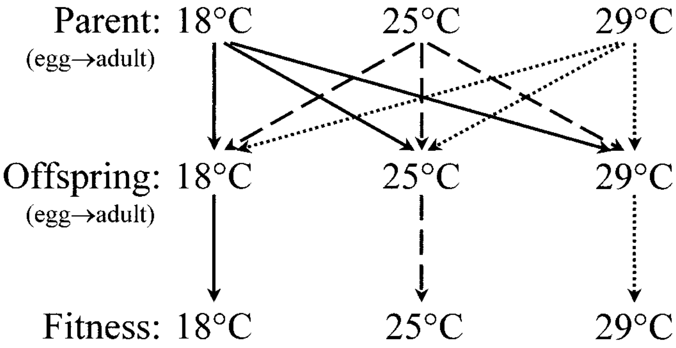

FIG. 2. Experimental design showing the temperature treatments for the parental and offspring (F_1) generations. Fitness of the F_1s was scored using the method of Mueller and Ayala (1981) by tallying the F_2 progeny.

TABLE 1. Predictions for the cross-generational effects hypotheses (Fig. 1). The terms in the table correspond to the main effects and contrasts with a single degree of freedom for the ordered factors in the linear model $\log(\lambda) = T_{off} + T_{par}$ (linear) + T_{par} (quadratic) + $(T_{off} \times T_{par}) + \epsilon$. ACG, adaptive cross-generational; CPB, colder parents are better; HPB, hotter parents are better; OPT, optimum parental temperature.

	Cross-Generational Hypothesis				
Term	Null	ACG	CPB	HPB	OPT
T_{par} (main effect)	ns	*	*	*	*
T_{par} (linear)	ns	ns	*(−)	*(+)	ns
T_{par} (quadratic)	ns	ns	ns	ns	*(−)
$(T_{off} \times T_{par})$	ns	*	ns	ns	ns

tions. Finally, the null hypothesis is that parental temperature (T_{par}) has no systematic effect on offspring fitness at various temperatures (T_{off}).

We adopt a strong inference approach (Huey and Berrigan 1996; Huey et al. 1999) to evaluate the relative merits of these hypotheses. The approach requires a 3 × 3 full-factorial design (Fig. 2). Specifically, we reared parents at 18°C, 25°C, or 29°C and reared and assayed fitness of their offspring at 18°C, 25°C, or 29°C. Then we used a factorial analysis of variance in which parental temperatures were treated as an ordered factor. All of the competing hypotheses (Table 1, Fig. 1, see Materials and Methods) can be tested by examining the strength and direction of the main effects, along with contrasts with a single degree of freedom on the ordered factors (orthogonal polynomials to test for linear and quadratic effects). For example, the OPT hypothesis would be supported if the quadratic contrast for parental temperature were significant and negative (Table 1). Of course, these hypotheses need not be exclusive (Huey et al. 1999), but our ANOVA approach allows us to rank in effect the relative impact of each hypothesis.

MATERIALS AND METHODS

Drosophila melanogaster (originally collected by L. G. Harshman near Davis, CA in 1996) were pulled from population cages (25°C, photoperiod 12:12 L:D) in two blocks, January 1997 and March 1998. Approximately 100 eggs were placed in each of nine bottles (30 ml of molasses/cornmeal medium) to found the parental (or P) generation. Three bottles were placed at each parental temperature (T_{par}: 18°C, 25°C, or 29°C, Fig. 2). When the adults eclosed, about 100 eggs from each parental temperature were placed in each of nine bottles, with three bottles incubated at each of the three offspring temperatures (T_{off}: 18°C, 25°C, or 29°C, Fig. 2) to form the offspring (or F_1) generation.

We used the per capita rate of population increase (λ) as an index of fitness and estimated it using Mueller and Ayala's (1981) Type 2 method (serial transfer). Approximately 24–48 h after eclosion, five F_1 males plus five F_1 females ($N = 10$) were transferred to a fresh bottle with 30 ml of medium and incubated at T_{off} for 1 week. Four replicates for each T_{par}-T_{off} combination were set up in the 1997 block and five in the 1998. At the end of the first week, we counted the survivors in the F_1 generation and cleared the bottles. The bottles were then returned to the incubator. In each subsequent week, the living adults (F_2) within each bottle were counted (Y_i). Those flies were discarded and the cleared bottle was returned to the incubator. Counts were made for three weeks for the 25°C and 29°C treatments and for five weeks for the 18°C, because of the prolonged development time at lower temperatures.

Counts from each week were transformed such that $a_i = Y_i/N^*$ where N^* was the initial number of flies in the bottle. Fitness (λ) was estimated by the largest positive eigenvalue of the equation $N_t = a_1N_{t-1} + a_2N_{t-2} + \cdots + a_tN_0$. Mueller and Ayala (1981) explain the methodology and its justification. No significant effect of block on fitness was detected ($P > 0.70$), so the data from 1997 and 1998 were pooled for all subsequent analyses.

Fitness estimates for each population were analyzed using a linear model: $\log(\lambda) = T_{off} + T_{par}$ (linear) + T_{par} (quadratic) + $(T_{off} \times T_{par}) + \epsilon$, where T_{par} is treated as an ordered factor allowing linear and quadratic contrasts with a single degree of freedom using orthogonal polynomials (Huey et al. 1999). The sign and significance of the various coefficients inform the significance of the hypothesis at risk (Table 1, Fig. 1). For example, if T_{par} (linear) is significant and positive, then the HPB hypothesis would be supported; or if T_{par} (quadratic) is significant and concave downward, then the OPT hypothesis would be supported.

RESULTS

Offspring fitness was significantly influenced by parental temperature (Tables 2, 3; Fig. 3). Specifically, fitness in-

TABLE 2. ANOVA of cross-generational acclimation effects. T_{off} and T_{par} are ordered factors; the linear and quadratic effects are tested using orthogonal polynomial contrasts. Fitness values (λs) were log transformed prior to analysis.

	df	MS	F	Pr(F)
T_{off}	2	36.770	4578.785	<0.001
T_{par}	2	0.149	18.556	<0.001
T_{par} (linear)	1	0.290	36.081	<0.001
T_{par} (quadratic)	1	0.008	1.031	0.313
$T_{off} \times T_{par}$	4	0.016	1.948	0.112
Residuals	72	0.008		

TABLE 3. Least squares means ± standard deviation for parental temperature effects on fitness, development time, and early fecundity. Post hoc comparisons were conducted using Tukey's method for simultaneous confidence intervals. Values with different letters are significantly different at $P < 0.05$.

T_{par}	N	λ (week^{-1})	Development time (week)	Fecundity (no. adults)
18°C	27	16.62 ± 1.088^{a}	1.87 ± 0.053^{a}	463 ± 53.5^{a}
25°C	27	17.88 ± 1.050^{b}	1.90 ± 0.075^{a,b}	468 ± 46.5^{a}
29°C	27	19.28 ± 1.132^{c}	1.83 ± 0.077^{c}	456 ± 74.1^{a}

creased linearly with T_{par} from 18°C to 29°C as evidenced by the significant linear contrasts (Table 2). The quadratic component of T_{par} was not significant. Neither of the interactions of T_{off} with either the linear or quadratic components of T_{par} was significant. A comparison of paired confidence intervals (Tukey's method, Table 3) reveals that fitness (λ) was highest for flies with parents reared at 29°C, intermediate for those with 25°C parents, and lowest for those from parents at 18°C (Table 3).

Our experiments were not designed to determine the specific life-history shifts underlying the observed effects of parental temperature on offspring fitness, however, we can use our data to explore this issue, albeit crudely and indirectly. Specifically, we can estimate whether parental temperature might influence either the early fecundity of offspring (F_1) or the development time of their grandoffspring (F_2). Early fecundity per bottle is simply the cumulative number of surviving adults removed from the bottle in the two weeks after the F_1 parents were removed. Mean development time of F_2 progeny of each bottle in the fitness assays is estimated by multiplying the number of surviving F_2 adults by the number of weeks since the F_1 parents were removed from the bottle. Both measurements are crude; nevertheless, they do reveal important differences among the parental temperature treatments. Flies with parents that developed at 29°C produced offspring that developed significantly more rapidly than did flies with parents at 18°C or 25°C (Table 3). Parental temperature had no significant effect on early fecundity (Table 3). Ultimately, of course, these patterns should be validated in direct experimental tests.

Within each parental temperature treatment, offspring fitness increased with test temperature (Fig. 3). Thus, flies at T_{off} of 25°C and 29°C consistently had higher fitness than those reared at 18°C (Table 2, Fig. 3). This undoubtedly results from the shorter development time of the F_2 offspring at the higher temperatures, relative to those developing at 18°C.

DISCUSSION

Parental thermal regime significantly influenced offspring fitness in *D. melanogaster*. Parents reared and living at 29°C produced offspring with higher average fitness than did parents reared at 25°C, which in turn produced offspring with higher fitness than did parents from 18°C (Table 3, Fig. 3). This pattern supports the HPB hypothesis. Moreover, it clearly contradicts an ACG hypothesis (Table 1, Fig. 1), which predicts that fitness is maximal when offspring live in the same environment as that of their parents (Mousseau and Dingle 1991; Leroi et al. 1994; Huey et al. 1999).

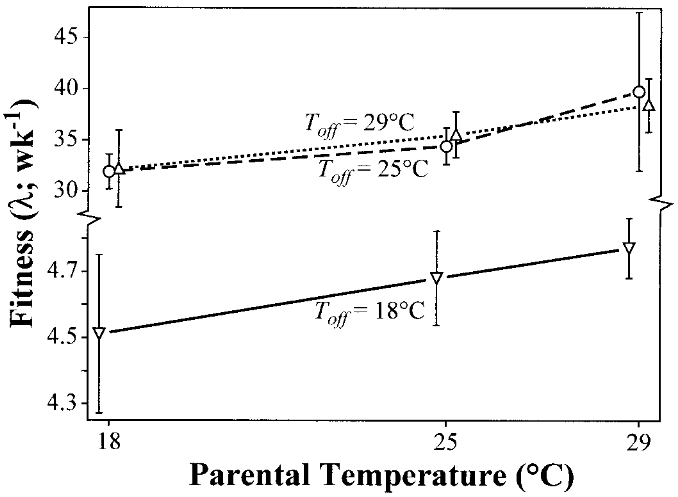

FIG. 3. Fitness (λ) of flies developing at T_{off} = 18°C, 25°C, or 29°C as a function of parental temperature (T_{par}).

What mechanism might explain why hotter parents are better? Mueller and Ayala's (1981) fitness assay is especially sensitive to rapid development and early fecundity. Thus, females whose parents were reared at high temperature may have greater early fecundity than do females whose parents were reared at low temperature or they may produce offspring (thus grandoffspring of the parental flies) that develop more rapidly. We found that the F_2 offspring of flies whose parents came from the high-temperature treatments completed development more rapidly than did the offspring of flies whose parents were reared at 18°C or 25°C. However, F_1 flies among the parental treatments did not differ significantly in early fecundity (Table 3).

Several insect studies support the general pattern that hotter is better in terms of developmental acclimation across generations. Using a partial-factorial design, Zamudio et al. (1994) found that male *D. melanogaster* derived from parents reared at 25°C better defended their territories at a high temperature than did males from parents reared at 18°C. Groeters and Dingle (1988) found that milkweed bugs from parents reared at 27°C attained reproductive maturity more rapidly than bugs from parents reared at 23°C.

The effects of parental temperature are likely to be complex and to affect diverse traits in diverse ways. Even though larger body size is often associated with higher fitness in *Drosophila* and other insects (e.g., Ewing 1964; Hoffmann 1987a,b; Partridge et al. 1987; Wilkinson 1987; Santos et al. 1988), individuals with a reduced size resulting from developmental and cross-generational effects of higher temperature (Zamudio et al. 1994; Crill et al. 1996) seem to have higher fitness than larger flies from low-temperature treatments. Perhaps high-temperature parents produce offspring that are physiologically more robust and more resistant to diverse stresses (Feder 1996). For example, Crill et al. (1996) showed that *D. melanogaster* offspring from parents grown at 25°C, despite their relatively small mass, had a higher knockdown temperature than did flies from parents grown at

18°C. In contrast, several studies (reviewed in Huey et al. 1995) have found that within- and between-generation effects of high temperature generally depress early fecundity. More research is needed to understand the interactions between life history, physiology, and morphology.

Using a similar factorial design and analysis, Huey et al. (1999) reanalyzed published data from several researchers to test competing hypotheses regarding developmental temperature effects on fitness. How well do those developmental temperature patterns compare with those observed here for parental temperatures? In the present experiments, the data exclusively support the HPB hypothesis. Previous experiments on developmental temperature acclimation generally supported the optimal developmental temperature hypothesis (Cohet and David 1978; Zamudio et al. 1994; Huey et al. 1999). Nevertheless, a few experiments showed limited support for selective advantage of higher temperatures (*Volvox* swimming speed: Herron 1996; *D. melanogaster* longevity: Zwann et al. 1991; *D. melanogaster* flight power output: Barnes and Laurie-Ahlberg 1986). Thus, developmental and cross-generational effects of temperature are not always parallel. However, both kinds of effects are clearly inconsistent with the common adaptive prediction that acclimation to a given environment enhances fitness.

In conclusion, parental temperatures have diverse and complex effects on offspring in *D. melanogaster* (Huey et al. 1995; Crill et al. 1996), but having hot parents seems to maximize offspring fitness. We suggest several ways in which this research can be extended. First, one could design a factorial experiment that separately manipulates parental, developmental, and adult thermal environments (as per Huey et al. 1995), and then directly estimate fitness over a range of F_1 ages (P. Gibert, R. B. Huey, and G. W. Gilchrist, unpubl. ms.) This would enable one to determine whether parental and developmental effects decay as an individual acclimates to its adult environment. Second, to make the experiments ecologically more realistic, one might use fluctuating, rather than constant, thermal regimes (Bradshaw 1980; Brakefield and Mazzotta 1995). In any case, further attention to the complex effects of temperature on parents and development definitely seems warranted.

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